Archives of Gerontology and Geriatrics 56 (2013) 75–79

Contents lists available at SciVerse ScienceDirect

Archives of Gerontology and Geriatrics

journal homepage: www.elsevier.com/locate/archger

CD36 expression in the brains of SAMP8

Bin Wu [a,b], Masaki Ueno [a,*], Takashi Kusaka [c], Takanori Miki [d], Yukiko Nagai [e], Toshitaka Nakagawa [e], Kenji Kanenishi [f], Naohisa Hosomi [g], Haruhiko Sakamoto [a]

[a] Department of Pathology and Host Defense, Faculty of Medicine, Kagawa University, Kagawa 761-0793, Japan
[b] Department of General Practice, First Affiliated Hospital of China Medical University, San Hao Road 36, Shenyang 110003, China
[c] Department of Maternal Perinatal Center, Faculty of Medicine, Kagawa University, Kagawa 761-0793, Japan
[d] Department of Anatomy and Neurobiology, Faculty of Medicine, Kagawa University, Kagawa 761-0793, Japan
[e] Department of Life Sciences Research Center, Faculty of Medicine, Kagawa University, Kagawa 761-0793, Japan
[f] Department of Perinatology and Gynecology, Faculty of Medicine, Kagawa University, Kagawa 761-0793, Japan
[g] Department of Clinical Neuroscience and Therapeutics, Hiroshima University Graduate School of Biomedical Sciences, Hiroshima, Japan

ARTICLE INFO

Article history:
Received 15 June 2012
Received in revised form 14 July 2012
Accepted 21 July 2012
Available online 9 August 2012

Keywords:
CD36
SAMP8
Oxidative stress

ABSTRACT

SAMP8, senescence accelerated mice with age-related deficits in memory and learning, are known to show age-related increases of amyloid precursor protein (APP) and immunopositivity for amyloid-β (Aβ) proteins, and moreover to be under elevated oxidative stress. The elevated expression of class B scavenger receptor CD36, which is the receptor of oxidized LDL and also one of efflux transporters of Aβ proteins in the cerebral vessels, is thought to mediate free radical production in cerebral ischemia and induce oxidative stress. Accordingly, by real-time quantitative reverse transcriptase-polymerase chain reaction (RT-PCR), Western blotting, and immunohistochemical techniques, we examined whether the expression of CD36 was increased in the brains of 10–12-week-old SAMP8 with elevated oxidative stress. Ten to 12-week-old SAMR1 mice were used as controls without the features. The gene and protein expression of CD36 was significantly higher in the brains of SAMP8 than those of SAMR1. Confocal microscopic examination revealed that the CD36 immunoreactivity was seen in the cytoplasm of endothelial cells and F4/80-positive perivascular cells of the brains. These findings indicate that the expression of CD36 in the brains of SAMP8 is increased compared with that of SAMR1.

1. Introduction

The senescence-accelerated mouse (SAM) is a general term for "accelerated senescence prone" (P-series; SAMP) and "accelerated senescence resistant" (R-series; SAMR) mice (Takeda et al., 1981). Among SAMP, SAMP8 mice have a short life span like the other SAMP mice, reveal some senescence-accelerated symptoms, and show a remarkable age-related deterioration in the ability of memory and learning in passive and active avoidance responses (Miyamoto et al., 1986). Previous papers showed that the expression of amyloid precursor protein (APP) and its mRNA was increased with aging in the brains of SAMP8 (Morley et al., 2000; Takemura et al., 1993). Recently, some papers showed that amyloid-β (Aβ) granules accumulated in the hippocampus of SAMP8 and that the number of the granules was increased with aging (del Valle et al., 2010, 2011; Manich et al., 2011).

It has been suggested that vascular Aβ receptors in endothelial cells transfer perivascular Aβ proteins into circulation and thus contribute to the clearance of Aβ from the brain (Zlokovic, 2004). Both the low-density lipoprotein receptor (LDLR) and the LDLR-related protein 1 (LRP1) may act as Aβ receptors (Abdulkarim & Hameed, 2006; Fryer et al., 2005; Sagare et al., 2007). LRP1 is a member of the LDLR family and functions both as a multi-functional scavenger and signaling receptor and as a transporter and metabolizer of cholesterol and apolipoprotein E (ApoE)-containing lipoproteins (Herz & Marschang, 2003). LRP1 binds both ApoE/Aβ complexes and Aβ and regulates their clearance from brain to blood (Donahue et al., 2006; Shibata et al., 2000; Zlokovic, 2004). Besides the LDLR family, some other potential Aβ-binding receptors have been identified. P-glycoprotein (Lam et al., 2001), scavenger receptor CD36 (Coraci et al., 2002), the formylpeptide receptor-like-1 (FPRL1) (Le et al., 2001), and the transmembrane APP itself (Lorenzo et al., 2000) also function as Aβ receptors. We previously investigated the expression of P-glycoprotein, LDL receptor, and LRP1, all of which were efflux transporters of Aβ proteins, and reported that the expression of P-glycoprotein and LDL receptor but not LRP1 was increased in the brains of SAMP8 compared with that of SAMR1 (Wu, Ueno, Kusaka, et al., 2009; Wu,

* Corresponding author at: Department of Pathology and Host Defense, Faculty of Medicine, Kagawa University, 1750-1 Ikenobe, Miki-cho, Kita-gun, Kagawa 761-0793, Japan. Tel.: +81 87 891 2115; fax: +81 87 891 2116.
E-mail address: masaueno@med.kagawa-u.ac.jp (M. Ueno).

0167-4943/$ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.archger.2012.07.007

Ueno, Onodera, et al., 2009). In addition, we reported that the expression of RAGE, an influx transporter of Aβ proteins, was decreased in the brains of SAMP8 compared with that of SAMR1 brains (Wu, Ueno, Onodera, et al., 2009).

CD36 belongs to the class B scavenger receptor family, which includes the receptor for selective cholesteryl ester uptake, scavenger receptor class B type I (SR-BI), and lysosomal integral membrane protein II (Febbraio, Hajjar, & Silverstein, 2001). CD36 is a surface glycoprotein and is localized in lipid rafts of plasma membrane and in mitochondria (Bonen et al., 2004; Campbell et al., 2004; Roepstorff, WulffHelge, Vistisen, & Kiens, 2004). In addition, CD 36 colocalizes with caveolin-1 in specialized plasma membrane microdomains known as caveolae (Lisanti et al., 1994). These cholesterol- and sphingolipid-enriched structures may serve to concentrate signaling molecules and facilitate the integration of signaling cascade. Multiple lines of evidence indicate that caveolae serve an integral role in the trafficking of cholesterol in cells. Oxidized LDL (oxLDL) depletes the endothelial cell caveolae of cholesterol, resulting in displacement of endothelial nitric oxide synthase and an altered response to acetylcholine (Uittenbogaard, Shaul, Yuhanna, Blair, & Smart, 2000). These disruptive effects of oxLDL are mediated by CD36 and can be blocked by interaction of SR-BI with HDL, which prevents the cholesterol depletion of caveolae. Thus, CD 36 has a role in the regulation of caveolar function. It is known that CD36 expression is broad and seen in microglia, macrophages, microvascular endothelium, adipocytes, dendritic cells, platelets, and cardiac, skeletal and smooth muscle cells (Febbraio, Guy, & Silverstein, 2004). CD36 recognizes a multitude of ligands, including oxLDL, long-chain fatty acids, thrombospondin-1, fibrillar Aβ, and the membrane of cells undergoing apoptosis (Febbraio et al., 2004; Hirano et al., 2003; Medeiros et al., 2004). In addition, some findings suggest that the expression of CD36 is involved in the pathogenesis of cerebral ischemia with reactive oxygen species (ROS) production (Cho et al., 2005). The increased ROS production is suggested to contribute to the pathogenesis of the SAMP8 brain (Alvarez-Garcia et al., 2006; Sato et al., 1996; Yasui et al., 2003). Accordingly, we examined whether the expression of CD36 is increased in the brains of SAMP8 with elevated oxidative stress, and whether CD36 could be a therapeutic target to brain injury with the elevated oxidative stress.

2. Materials and methods

The experimental protocols for animal care were in compliance with institutional guidelines of Kagawa University. Ten to 12-week-old male SAMP8 ($n = 10$) and SAMR1 ($n = 10$) mice (purchased from Japan SLC, Inc., Japan) weighing 25–35 g were used. All efforts were made to minimize the number of animals used and their suffering. The animals were anesthetized with diethyl ether and then used for several experiments.

For real-time RT-PCR and Western blot analyses, mice were perfused transcardially with phosphate-buffered saline (PBS). Their brains were removed and stored at −80 °C. Extraction of total RNA and reverse transcription of the total RNA were performed as reported previously (Huang et al., 2004). To quantify gene expression, TaqMan real-time quantitative PCR was performed with the ABI PRISM 7700 Sequence Detection System using Assays on-Demand Gene Expression probes of CD36 (Applied Biosystems, Foster, CA, USA). The PCR cycling conditions for all samples were as follows: 50 °C, 2 min for AmpErase UNG activation; 95 °C, 10 min for AmpliTaq Gold activation; and 50 cycles for melting (95 °C, 15 s) and annealing/extension (60 °C, 1 min) steps. Each sample was run in duplicate. The comparative threshold cycle method (Applied Biosystems) was used to calculate gene expression in each sample relative to the value observed in control samples using glyceraldehydes 3-phosphate dehydrogenase (GAPDH) as a control for normalization among samples. For Western blot analysis, brain tissues were homogenized in lysis buffer containing 10 mmol/L Tris buffer containing 2 mmol/L phenylmethylsulfonyl fluoride, and 10 mmol/L N-ethylmaleimide with a protease inhibitor. The extracts were solubilized in a Tris/glycine/sodium dodecyl sulfate (SDS) sample buffer in the presence of 5% 2-mercaptoethanol (Bio-Rad) and heated at 95 °C for 5 min. Protein extracts (40 μg) were then separated by 10% SDS polyacrylamide gel electrophoresis (SDS-PAGE). The separated proteins were transferred from gel to nitrocellulose membranes and blocked in a blocking solution (5% dry milk in PBS) for 1 h. The membrane was incubated with a primary rabbit antibody for CD36 (1:500, ProteinTech Group, Inc., Chicago, IL, USA) at 4 °C overnight. The membranes were then incubated with HRP-labeled anti-rabbit IgG antibody (1:5000, Amersham Biosciences, Buckinghamshire, UK) for 1 h at room temperature (RT). The proteins were visualized on enhanced chemiluminescence film (Hyperfilm; Amersham Biosciences). Finally, the blots were reprobed using an antibody against actin (1:2000, Epitmics, Inc., Burlingame, CA, USA). Data are expressed as the relative differences among samples after normalization to actin expression. The immunoreactive bands were quantified by densitometric analysis (NIH image software).

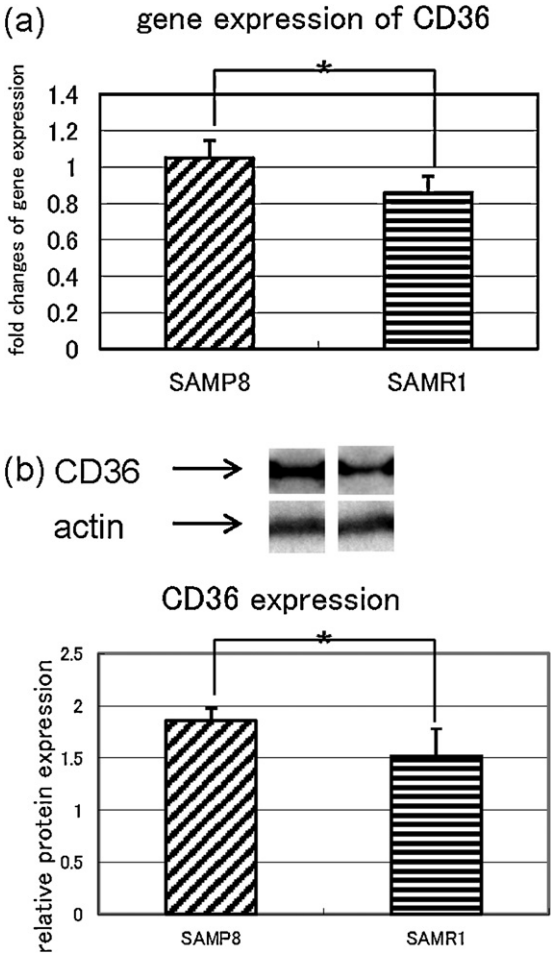

Fig. 1. (a) mRNA expressions of CD36 after normalization to GAPDH expression in the brains of SAMP8 (bar indicated by oblique line) ($n = 6$) and SAMR1 (bar indicated by horizontal line) ($n = 6$) mice are shown. The gene expression of CD36 is significantly higher in the SAMP8 brains than that of SAMP8 (*, $p < 0.05$). (b) Western blotting analyses shows representative bands of CD36 and actin of SAMP8 and SAMR1 brains. The protein expression of CD36 is significantly higher in the SAMP8 brains than that of SAMR1 (*, $p < 0.05$).